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13. Abstract (Maximum 200 Words) <small>(abstract should contain no proprietary or confidential information)</small> Recently breast cancer resistance protein (BCRP) has been found to be a frequent cause of MDR by causing increased efflux of a wide variety of cytotoxic drugs. Although it has been shown that transfection of BCRP into breast cancer cell line MCF7 caused drug resistance, it has also been found that the drug resistance level of these cells were much lower than that of the drug-selected cells. Thus, there must be other drug resistant mechanisms in the drug selected MCF7/AdrVp cells. This study is designed to test this concept. Specifically, we plan to achieve the following objectives using proteomics technology: (a) to compare protein profiles between MCF7 and MCF7/AdrVp cells using two-dimensional gel analysis, (b) to identify the proteins of different levels between the two cell lines using MALDI-TOF mass spectrometry analysis, (c) to confirm the different level of the identified proteins using western blot, and (d) to test the role of these proteins in mediating MDR using MTT assay. The information and probes obtained from this study will help us understand the molecular mechanism of drug resistance in breast cancer cells. This work may also help us discover new therapeutics for treating drug resistant breast tumors.				
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INTRODUCTION

The use of anticancer agents in appropriate combinations has led to major improvements in the treatment of malignant tumors. Previously fatal diseases, such as Hodgkin's disease, are now curable while others, such as breast cancer, can undergo remission. Resistance to chemotherapy frequently occurs in breast cancers and is a major obstacle to successful breast cancer treatment. Studies with tumor cell lines such as MCF7 have revealed that multidrug resistance (MDR) can develop and thus cause chemotherapy failure. Advances in elucidating the molecular basis of the MDR phenotype indicate that expression of P-glycoprotein (Pgp) and multidrug resistance protein 1 (MRP1) is a frequent cause of MDR in human breast cancers (Ambudkar et al., 1999). Recently, another membrane protein, breast cancer resistance protein (BCRP), has also been found to be a frequent cause of MDR (Doyle et al., 1998)-(Miyake et al., 1999). Pgp, MRP1 and BCRP all belong to the ATP-binding cassette transporter superfamily (Dean et al., 2001). Cancer cells over-expressing Pgp, MRP1, or BCRP have an increased ability to efflux a wide variety of cytotoxic drugs and, therefore, can survive chemotherapy (Gottesman et al., 2002).

BODY

This progress report is for a concept award. In the original application, we proposed to accomplish the following objectives: (a) to compare protein profiles between MCF7 and MCF7/AdrVp cells using two-dimensional gel analysis, (b) to identify the proteins of different levels between the two cell lines using MALDI-TOF mass spectrometry analysis, (c) to confirm the different level of the identified proteins using western blot, and (d) to test the role of these proteins in mediating MDR using MTT assay.

We have accomplished most of our studies as planned. Firstly, a regular SDS-PAGE was performed and a protein of 275 kDa was found over-expressed (see Figure 1 in the poster appended). This protein was later identified to be fatty acid synthase by MALDI-TOF mass spectrometry (see Table 1 in the poster appended). A two dimensional gel electrophoresis was then conducted on the cell lysates prepared from the parental drug sensitive MCF7 cells and the drug-selected MCF7/AdrVp3000 cells and 17 protein spots were found to be differentially expressed between the two cell lines (see Figure 2 in the poster appended) and were identified by MALDI-TOF mass spectrometry (see Table 1 in the poster appended). We then confirmed the expression level of some of these proteins using western blot and real time RT PCR (see Figures 4 and 5 in the poster appended). We are currently in the process of testing whether the altered expression of these proteins plays any role in drug resistance in breast cancer cells.

KEY RESEARCH ACCOMPLISHMENTS

1. Seventeen proteins were identified which have differential expression levels between the drug sensitive parental MCF7 and the drug resistant MCF7/AdrVp3000 cells.
2. The differential expression levels of some of these proteins were confirmed by western blot and/or real time RT PCR.

REPORTABLE OUTCOMES

1. Liu, Y.; Liu, H.; Zhang, J.-T. Proteomic analysis of drug resistant breast cancer cell line MCF7/AdrVp3000 (2004). Proceedings of American Association of Cancer Research 45 (<http://aacr04.agora.com/planner/displayabstract.asp?presentationid=3465>).

CONCLUSIONS

In conclusion, at least 17 proteins have altered expression level in the drug selected MCF7/AdrVp3000 cells compared with the parental drug sensitive MCF7 cells. This observation suggests that other mechanisms are likely also responsible for drug resistance of MCF7/AdrVp3000 cells in addition to the known the increased drug efflux due to elevated expression of BCRP. We are currently testing these possibilities.

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Proteomic Analysis of Drug

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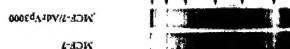


Figure 1. SDS-PAGE analysis of MCP-7 and MCP-4 cells. MCP-7 and MCP-4 cells were harvested at 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.5% NP-40, 20 mM EDTA, 2 mM PMSF, 1 mM DTT, 1 mM NaVO₃, and 1 mM SDS by sonication. After centrifugation at 10,000g for 10 min, 20 μ g proteins from the supernatant were separated by SDS-PAGE and stained for convenience. Molecular weight standards (Sigma) are indicated on the left.

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INTRODUCTION A major obstacle in the efficient chemotherapy of human cancer is the intrinsic or acquired multidrug resistance (MDR) to both hydrostatic and cytotoxic drugs. To study the mechanisms of drug resistance, many drug resistant cell lines have been developed *in vitro* by selecting with various agents. A drug resistant breast cancer cell line, MCF-7/AdV-p3000, was isolated from parental MCF-7 cells by stepwise selection with adriamycin in the presence of P-glycoprotein inhibitor verapamil. MCF-7/AdV-p3000 displays an AdV-dependent reduction in the intracellular accumulation of anthracycline anticancer drugs in the absence of over-expression of known multidrug resistance transporters such as P-glycoprotein or the multidrug resistance-associated protein. A half ABC transporter, ABCG2, was shown to be over-expressed in this cell line. MCF7 cells transfected with ABCG2 cDNA showed similar profile but with a much reduced level of drug resistance when compared with that of MCF-7/AdV-p3000 cells. In addition, two non-drug resistance proteins, NCA-19 gene and NCA-90 (non-specific cross-reacting antigen), were also identified to be highly expressed in MCF-7/AdV/p cells. a derivative cell line from the early steps of selection. In an attempt to investigate whether other genes may have been selected in Adriamycin in MCF-7/AdV-p3000 cells but absent in the parental MCF-7 cells, we applied the proteomic approach which combines two-dimensional gel electrophoresis and matrix-assisted laser desorption/ionization (MALDI) mass spectrometry to compare the global protein profile and to identify the proteins differentially expressed between MCF-7 and MCF-7/AdV-p3000 cells.

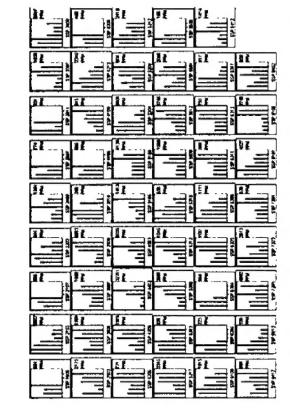


Figure 3. Quantitative levels of protein spots with differential expression between MCF-7 and MCF-7/Adrv15000 cells. Individual bars represent the protein quantity value of each gel of three different gels. The left three bars correspond to MCF-7 and the right three bars correspond to MCF-7/Adrv15000 cells. SSP numbers represent the spot number of each protein. The value in y-axis is depicted as parts per million (ppm) determined using PDQuest gel analysis software.

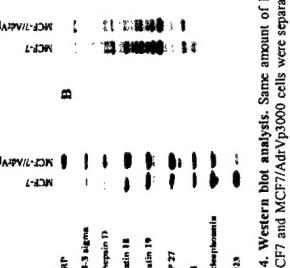
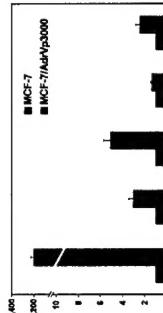
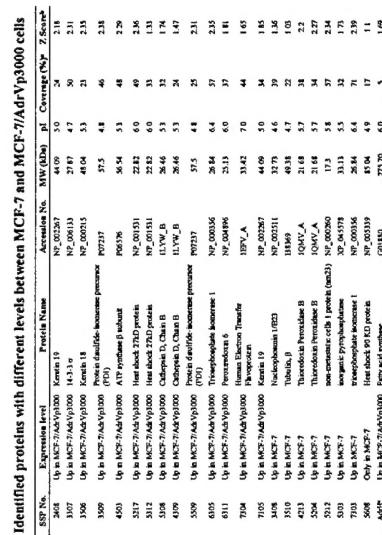


Figure 4. Western blot analysis. Same amount of lysates from MCF7 and MCF7/AdVp100 cells were separated by SDS-PAGE followed by western blot analysis (A) for ABCG2 (BCRP), 14-3-3 α , cathepsin D, keratin 18, keratin 19, protein disulfide-isomerase precursor (PDI), heat shock protein (HSP70), nucleophosmin 1, and NFM23 or for staining with commassie blue (B).



Total RNA isolated from MCF-7 and MDV-1/dvP3/000 cell lines were reverse transcribed using AMV reverse transcriptase and Oligo(dT)₁₂₋₁₈ primers. Real time quantitative PCR was carried out using gene specific primers. Relative mRNA levels were measured using SYBR Green and calculated in the fold change ($2^{-\Delta\Delta C_t}$) relative to MCF-7 cells after normalized by the internal control, GAPDH.



Protein spots from triplicate gels were excised, digested with 55 mM Edman reagent and analyzed by MALDI-TOF mass analysis. The measured peptide mass profiles were then compared with the theoretical peptide masses using Profound search engine and NCBI database for protein identity.

- These proteins with differential expression between the two cell lines may be responsible in part for the drug resistance blot and/or real time quantitative RT-PCR.